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PLANT PROTECTION BULLETIN

A PUBLICATION OF THE WORLD REPORTING SERVICE ON PLANT DISEASES AND PESTS

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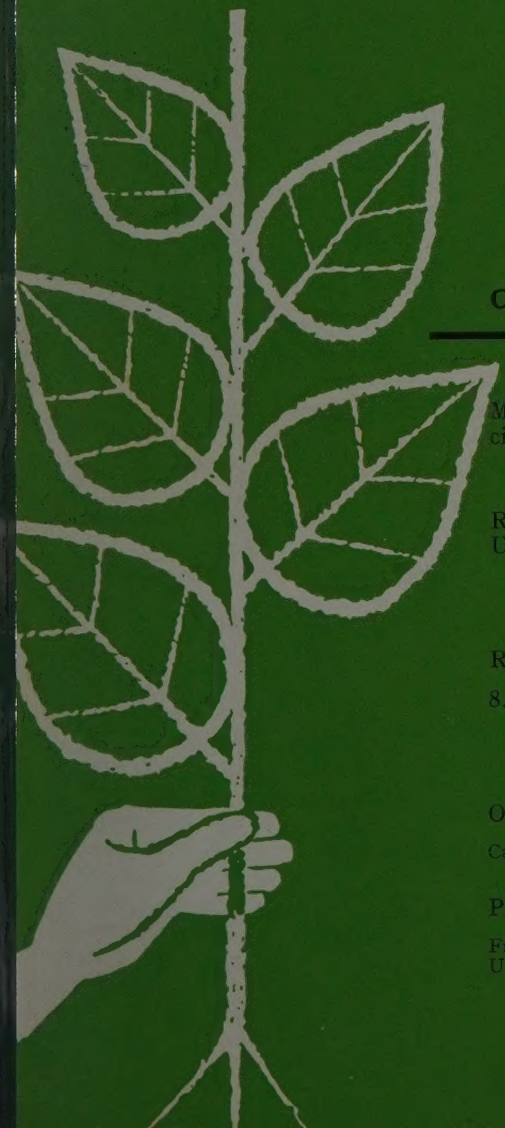

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is issued as a medium for the dissemination of information received by the World Reporting Service on Plant Diseases and Pests, established in accordance with the provisions of the International Plant Protection Convention, 1951. It publishes reports on the occurrence, outbreak and control of pests and diseases of plants and plant products of economic significance and related topics, with special reference to current information. No responsibility is assumed by FAO for opinions and viewpoints expressed in the Bulletin.

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FAO PLANT PROTECTION BULLETIN

A PUBLICATION OF THE WORLD REPORTING SERVICE ON PLANT DISEASES AND PESTS

Mathematical Treatment of the Rate of Loss of Pesticide Residues [1]

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Many entomologists, while considering the present difficulties in insect control, will let their thoughts go back to a very similar crisis some quarter of a century ago. In the late 1930s resistance of scale insects to cyanide was widespread and the slow but cumulative ill effects of spray oils on citrus and other trees had become obvious. Codling moths had become so tolerant to arsenic that apple trees had to be literally whitewashed with lead arsenate and practical control still was not secured by such heroic treatment in some districts. The development of young trees and cover crops was seriously affected by the accumulation of lead and arsenic in orchard soils. Further, American fruits were refused in certain European markets because of excessive residues, and the United States Food and Drug Administration was imposing annually decreasing tolerances for arsenic and lead which could not be met in the most seriously affected districts by any cleaning process short of peeling the fruit.

If the situation just described is extended from a few insects on a few crops to many insects on many crops, one will arrive at a fair picture of the present difficulties of insect control. The insecticides are different but the problems are the same and the role of the chemist has not changed. Thus substitutes for failing insecticides were sought and tried, e.g., phenothiazine and xanthone for lead arsenate to control the codling moth and similar pests. The dinitro compounds were found to be good against mites and aphids but of little use for control of cyanide – resistant scales and their phytotoxicity presented a constant hazard.

The point to be emphasized is that chemists were as busy determining deposits and residues at that time as they are at present. The apple industry of Washington and Oregon set up laboratories in which apples were analyzed after each spraying to ascertain that a sufficiently heavy deposit of lead arsenate had been applied. Close to harvest time repeated analyses were made, and after wiping or washing had become common practice, analyses were made before and after to forestall seizure of the fruit. The same problems recur today.

The spectacular breakthrough in insect control that followed the introduction of DDT and other highly effective materials in the early 1940s was accompanied by a remarkable lapse of judgment on the part of entomologists, chemists and even toxicologists and public health workers. Since these new materials were all organic compounds whose insecticidal power was the result of molecular structure rather than of inherent toxicity of an atom, it was widely assumed that exposure to the atmosphere would rapidly result in degradation to harmless derivatives. Tolerances on food products should not be necessary. Since, in many cases, even careless application gave better control than former insecticides, there was no interest in determining deposit and its changes after application. Hence there was no need for the

[1] Contribution from Laboratory of Insect Physiology and Toxicology, University of California.

analyst, and chemical efforts were focused on finding new materials as more and more manufacturers sought a place in the booming insecticide trade.

The end of this period was foreshadowed by the discovery that houseflies had become resistant to DDT in Sweden by 1947 and in many other regions soon thereafter. The end came definitely when studies with animals revealed toxic effects from low intake of DDT and analyses of treated crops showed that it is a stubbornly persistent substance. The imposition of tolerances for several of the "wonder insecticides," following the 1950 hearings of the Food and Drug Administration, completed the cycle back to the status of the 1930s.

The immediate analytical problem was to determine if tolerances were exceeded as a result of current control programs and thousands of harvest samples were analyzed. This effort enabled the entomologists to adjust their programs until satisfactory control without excessive residue was attained or to reject the chemical for those uses in which the two objectives could not be met. Such harvest residue data are of great value but they provide no adequate basis on which changes in control practice can be made during the growing season nor for setting up a procedure to handle emergency situations such as an insect outbreak in an unusual area or on a crop which normally needs no treatment.

For these purposes information is required on the changes in the amount of insecticide during the interval from application to harvest and sometimes through whatever processing is used with the foodstuff. Since it is practicable to make analyses only at intervals during the period, a limited number of points relating residue with elapsed time are available from which to reconstruct the sequence of events. If only one application is made and linear axes are used for time and amount, the points will define a curve decreasing from the initial deposit to some lower value at the time of last analysis.

Figure 1 shows data on DDT dust applied to collards at the rate of 30 pounds of 5 percent dust per acre near harvest time (2). Residues

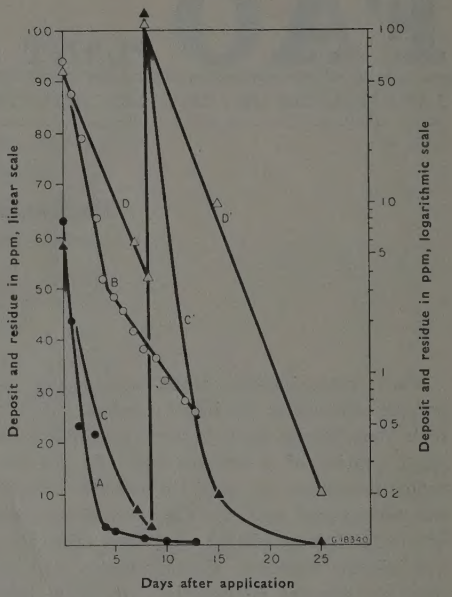

Figure 1. Changes in the amount of insecticides on plants after application. Lines A and B : DDT 5% dust applied at 30 lb per acre on collards ; A, plotted on linear scales ; B, plotted on semilog scales. Lines C, C', D, and D' : DDT wettable powder applied at 1 lb per acre on Iceberg lettuce ; C, single application, plotted on linear scales ; C', repeated application, plotted on linear scale ; D, single application, plotted on semilog scales ; D', repeated application, plotted on semilog scales.

are expressed in parts per million (ppm) by weight, and line A refers to the linear scale on the left and line B to the logarithmic scale on the right. The lines illustrate two important properties of many insecticide residues: (*a*) a rapid drop immediately after application; and (*b*) a much slower rate of loss later. It is obvious that the rates of loss are difficult to measure from the curve drawn to linear co-ordinates but the line on the semilogarithmic plot divides into two straight portions of quite different slopes. Repeated applications result in a saw-toothed line such as C in Figure 1, which represents the residues of DDT resulting from successive applications on Iceberg lettuce (10), plotted on the linear scales (dark triangles of line C) and on the semilog scales (open triangles of line D). The vertical line

at eight days indicates the increase in deposit due to the last application. It may be noted that the two parts of line D have almost the same slope. In all such cases of multiple treatment, the loss curves following each treatment may be handled independently. When the slope has been established by analyses at application time and at one or two intervals thereafter, these lines are useful in deciding when the residue will reach a level that requires another application.

Theoretically, it is reasonable that residues should decrease logarithmically, since the amount lost per unit time should be proportional to the total present at any time, provided all is equally exposed to weathering, degradation reactions, etc. This is the case for the initial period characterized by rapid loss of loosely adhering deposit by wind action, effect of rain or dew, etc. With materials which penetrate into the waxy covering of leaves or fruit or even into the deeper tissues of a plant, such mechanical losses are replaced by metabolic or other degrading processes which still occur in proportion to the amount of residue present. Naturally, while this second stage is occurring, external residue may still be leaving via mechanical or chemical action. In some cases absorbed insecticide will be protected in heavy wax or in oil glands and will persist for very long periods, as exemplified by parathion in citrus peel (1).

Instead of plotting residue on the logarithmic scale, the reverse method of plotting log time has been used by some writers (5, 15). Such plots give relatively straight lines for longer periods after application, i.e., near harvest time, but they have no theoretical basis and they suffer from the serious disadvantage that the time scale is shortened because of being expressed in logs. Hence it is difficult to estimate accurately the time required to reach any chosen value such as the tolerance for an insecticide on a certain crop. Also, the calculations to be given later for the effect of growth or other factors cannot be made in any simple way.

The straight lines corresponding to any of the stages of loss are all represented by the same form of equation, namely : log deposit = $k_1 t + \log k_2$ (Equation 1), in which k_1 is the slope of the regression line (always negative in sign) and k_2 is the apparent initial deposit obtained by extrapolating the line back to zero time. For the first stage of loss this k_2 value is usually equal or close to the actual initial deposit. For the second and third stages it is less. Since residue data have their greatest value in connection with the tolerance for the chemical on a particular crop, classification of chemicals

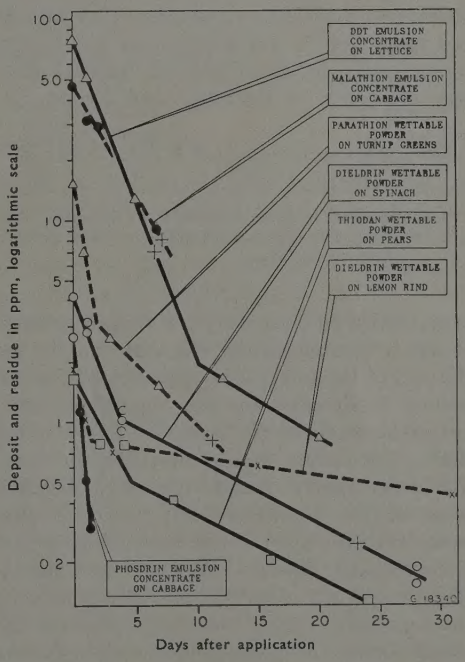

Figure 2. Changes in the amount of several insecticides on plants after application, based on the data given in Table 1.

is made best in terms of the line which crosses the tolerance level. This is indicated in Figure 2 by crossed lines for several cases. Table 1 gives the values of the constants k_1 and k_2 for the materials of Figure 2. For additional data on residues of 47 chemicals on the more important crops, reference may be made to the Final Report on Regional Project W-45 (9). It may be noted that the dimensions of k_1 are

TABLE 1. Values of the constants k_1 and k_2 and other data for insecticides shown in Figure 2

Insecticide	Formulation [b]	Dosage, actual	Crop	Constant k_1	Constant k_2 in ppm	Time interval, in days
DDT (10)[a]	25% EC	0.75 lb/acre	Lettuce	−0.15	79	0–10
				−0.04	4.3	10–20
Dieldrin (13)	25% WP	0.25 lb/acre	Spinach	−0.16	4.2	0–4
				−0.03	1.3	4–28
Dieldrin (7)	25% WP	9 lb/acre [c]	Lemon rind	−0.25	1.8	0–2
				−0.01	0.8	2–32
Malathion (14)	50% EC	20 oz/acre	Cabbage	−0.11	47	0–8
Parathion (14)	15% WP	2.4 oz/acre	Turnip greens	−0.35	15	0–2
				−0.06	3.9	2–7
Phosdrin (3)	EC	0.25 lb/acre	Cabbage	−1.0	3.8	0–2
Thiodan (4)	25% WP	0.5 lb/100 gal	Pears	−0.11	1.7	0–5
				−0.03	0.7	5–24

[a]) The numbers in parentheses refer to literature cited.
[b]) EC = emulsible concentrate; WP = wettable powder.
[c]) Excessive application.

logarithm (a pure number) and reciprocal days. Hence it is conveniently expressed as the reciprocal of the interval in days needed for the residue to decrease by one logarithmic unit, i.e., to a tenth of the value at any starting time. For k_2 any desired unit may be used. The usual ppm by weight leads to a negative slope of the line whenever growth occurs, even if there is no actual loss of pesticide. To avoid this, residues are sometimes expressed in weight per plant or fruit, in which event the log residue minus time line represents the actual decrease of residue, because of the factors mentioned earlier.

Table 1 shows that the values of k_1 correspond to what may be called fast, medium and slow rates of loss. A study of residue data for all available compounds shows that these values of k_1 may be grouped as: > 0.10 (TEPP, phosdrin, aramite, lindane, metacide, malathion on most crops, parathion on a few crops), > 0.01 and < 0.10 (most insecticides on most crops); < 0.01 (dieldrin and other chlorinated hydrocarbons in citrus rind, schradan in walnuts, parathion in olives). Such protected residues may persist for months. The formulation has an effect, especially upon the early rate of loss, but in general k_1 is independent of the dosage and initial deposit.

A useful property of residual pesticides is the time required for the deposit to be reduced to one half or any other given fraction of the original value. By rearranging Equation 1 and using $t_{1/2}$ to indicate the time for loss of half the calculated initial deposit, it follows that $t_{1/2} = \log 2/k_1 = 0.301/k_1$. Although there have been objections to the use of "half-life" with reference to insecticide deposits, their persistence is entirely analogous to that of the radioactive isomer of an element, and the term half-life is equally applicable. Surely the two situations will not be confused. As an example of calculation of half-lives from the above equation, the data for parathion on turnip greens in Table 1 may be used to give $0.301/0.35 = 0.9$ day for the first rapid loss and $0.301/0.06 = 5$ days for the rate of loss during the period when the tolerance of 0.8 ppm is reached. It may be noted that the time required to reach any fraction of the calculated original deposit may be calculated in a similar manner by use of the general relation: $t_{1/n} = \log n/k_1$, in which n

is the reciprocal of the fraction required. For example, $t_{1/10} = \log 10/k_1 = 1/k_1$.

One of the most important uses of residue data is the determination of the minimum interval required between application and harvest. This can be done from a plot such as Figures 1 and 2 if this is at hand, but it also may be calculated readily from a table of k_1 and k_2 values by use of the fundamental equation: log deposit $= k_1 t + \log k_2$. Letting $t_{tol} =$ time to reach the tolerance and again rearranging the equation: $t_{tol} = (\log tol/k_2)/k_1$. Since k_2 exceeds the tolerance in any useful case, it is simpler to change this to the form $t_{tol} = (\log k_2/tol)k_1$, with k_1 used as a positive number instead of its actual negative value.

In the previous discussion, methods for expressing the decrease in amount of a deposit have been considered without regard to the relative importance of various factors which affect the rate of change. Many of these are obvious, such as volatility and chemical stability of the toxicant, type of formulation, amount per acre or per plant, rainfall, temperature, air movement, and growth of the plant after application. Entomological literature contains many bits of data on these matters but few systematic studies have been made. The rates of loss of DDT from forage crops under influence of sunlight, wind or rain were measured by Hopkins *et al.* (8). Growth, weathering and formulation were considered by Sloan *et al.* (11) as factors in the loss of DDT and of parathion from lettuce, and Taschenberg and Avens (12) have reported recently on similar factors in the loss of DDT from grapes. A careful study of the removal of DDT deposits on bean and pear leaves by Dormal and Caussin (6) showed rapid loss with the first rainfall, decreasing thereafter to very low loss with long continued rain.

Growth of treated plants does not affect the actual amount of residue per individual fruit or other part of a plant, but it diminishes the ratio of weights, i.e., the contamination expressed in ppm. There is a considerable volume of data on plant growth in the botanical and horticultural literature. Growth patterns vary widely among different species of plants but during the active period growth is usually a logarithmic function of time and hence weights plotted on a log scale versus a linear time scale give a straight line of positive slope. Hence the difference $k_1 - k_g$ is the rate of loss of a residue corrected for effect of growth, and k_g/k_1 is the fraction of change due entirely to growth. Unfortunately, these simple relations do not apply near harvest time, for many plants or fruits cease to gain in weight as maturity approaches. Irrespective of the mode of change or lack of it, the effect of growth may be allowed for in any given time interval by multiplying the over-all rate of change by the ratio of initial to final weight for the period, i.e., rate of loss corrected for weight change $= r_1 \times w_1/w_2$.

In the case of a pesticide on a certain crop which is lost at a rate corresponding to $k_1 = 0.097$, the residue drops to one half in 3.2 days ($t_{1/2} = 0.301/0.097 = 3.2$ days). Should the weight of the crop increase at the rate of $k_1 = 0.041$, the residue in ppm will be halved because of growth alone in 7.3 days. Then the effect of all other factors is $0.097\text{-}0.041 = 0.056$, which corresponds to 5.3 days for a decrease to one half. If the effect of the other factors such as evaporation, rainfall, etc., can be isolated by suitable experiments, their separate effects can be calculated in the same manner. The effect of different formulations can be treated similarly. Further discussion of the consequence of growth and environmental conditions is given in the Project W-45 report (9).

In the cases mentioned previously, the analysis has been expressed in terms of the insecticide applied; no consideration was given to possible activation to derivatives more responsive than the original substance to the test used, nor to nontoxic derivatives that still respond to the test. Among modern organic insecticides, one or the other, or both of these changes are rather common; the thiophosphates may change to phosphates of greater inhibitory power toward enzymes (e.g., malathion→ malaoxon), and noninsecticidal degradation products are counted as active (nitrophenol from parathion responds to the Averill-Norris

test). In all such cases, it is necessary to determine what has happened by use of specific tests, chromatography, infrared, etc., before expressing the residue in terms of the original material and applying any method for expressing the rate of loss.

In the foregoing discussion, the use of semilog plots for expressing the change in amount of residues has been illustrated by insecticides on plants. It is, of course, not so narrowly limited, for the procedure is equally applicable to fungicides, weedkillers or any other chemical, and application may be made to nonliving surfaces, such as the walls of a house, or to the soil. Chemists will play a leading part in the future development of pest control and public health by the patient accumulation of accurate data on the factors that control the persistence of the chemicals used for these purposes.

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Ratoon-stunting Disease of Sugar Cane in the United Arab Republic

F. Nour-Eldin, M. A. Tolba, M. T. Elbanna and S. H. Farrage,[1] Plant Virus Research Unit, Ministry of Agriculture, P.O. Orman, United Arab Republic

Ratoon-stunting disease of sugar cane was first noticed in Egypt by Tysdal [2] during his visit in 1954. Later, Nour-Eldin [3] reported the disease to be widespread in the Kom Ombo area of Upper Egypt occurring both in varieties grown commercially, e.g., Co. 413 and N. Co. 310, and in imported varieties in experimental trials, e.g., P.O.J. 1030, Co. 281, Co. 290, Co. 419, P.O.J. 2878, 48 D. 12, 48 D. 57, 48 D. 26, 48 D. 53, 48 D. 10, 48 D. 110, 48 D. 69, 43 G. 61, 43 G. 47, 43 F. 35, 46 F. 3, 43 F. 89, E. 52, 48 E. 56, 48 I. 16, 48 F. 13, 40 F. 11, Q. 50.

Because sugar cane stalks infected by ratoon-stunting virus do not exhibit external symptoms, the survey with regard to the occurrence of this disease had to depend on internal symptoms. The characteristic histological symptom is a discoloration of vascular bundles restricted to the lower portion of the nodal region. The discoloration is distributed as dots and these are best seen on slicing lengthwise through the nodal region. This discoloration, ranging from orange to brick red, is very pronounced in the basal nodes and decreases gradually in those higher up. In several varieties, e.g., N. Co. 310, Co. 290, and P.O.J. 1030, the vascular discoloration is striking. In other varieties, such as Q. 50, the discoloration is pale and occurs in only a few vascular bundles.

Examination of the above-mentioned varieties revealed that 50 to 80 percent of the stalks examined were affected by these symptoms. Such high percentages suggest the possibility that this disease may be widely disseminated under conditions of sugar cane production in Egypt.

To evaluate the full impact of this disease on the nation's sugar economy, a study was initiated in February 1958. This paper presents the results of such studies, particularly as they bear on matters concerning transmission, pathological histology, and control.

Ratoon stunting was first recognized as a distinct virus disease in Queensland, where it was extensively investigated.[4]

Transmission trial

In March 1958, 20 sugar-cane cuttings of the N. Co. 310 variety exhibiting the characteristic internal symptoms of ratoon stunting were treated in an electric hot-water bath at 52°C for 1½ hours. The setts were taken from the basal parts of mature stalks. Each had three buds. Setts were planted in 20-inch clay pots immediately after treatment.

In May 1958, juice from infected stalks was extracted by means of a blender. The extracted juice was run through a bacteriological filter with a pore size of approximately 1.3 microns. The bacteria-free filtrate was used to inoculate stalks growing from the ten cuttings planted in March. Inoculum was introduced through an incision made at the base of the stalk below soil level; several drops of the bacteria-free juice were then applied to the inoculum. The

[1] The writers wish to express their gratitude to the staff of the Kom Ombo Agricultural Company, especially Dr. Y. El-Alayli, Mr. A.E. Ali, Mr. A. Khafaga and Mr. I. Saiid, for their generous co-operation and provision of facilities. Thanks are also due to Mr. F. Bishay of the Plant Pathology Section for his assistance in examining canes.

[2] Tysdal, H.M. 1954. Report to the Sugar and Distillation Company, Cairo. (Unpublished)

[3] Nour-Eldin, F. 1958. Report to the Ministry of Agriculture on virus diseases affecting sugar-cane cultivations in the Kom Ombo area. (Unpublished)

[4] Hughes, C. G. and D.R.L. Steindl. 1955. Ratoon-stunting disease of sugar cane. Queensland Bur. Sugar Exp. Sta. Tech. Com. 2.

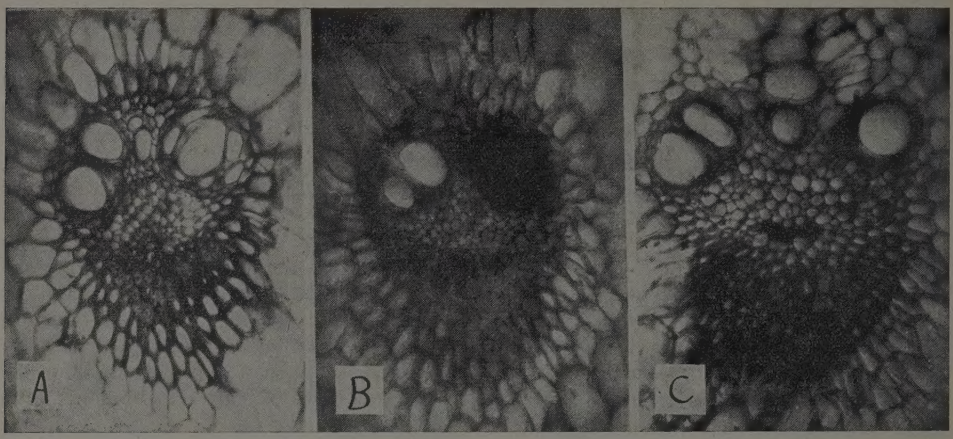


Figure 1. Histological changes in the vascular bundles of the stalks of sugar cane infected with ratoon-stunting virus, as shown in the cross sections of the lower part of the node. A. Healthy cane. B. Diseased cane, showing plugg of the wood vessels. C. Diseased cane, showing phloem necrosis.

same incisions were made in the noninoculated setts; here, however, distilled water was applied.

In September 1958, inoculated and noninoculated stalks were cut and examined for the presence of vascular discoloration in the basal nodes. The stalks were sliced both lengthwise and crosswise through the nodal region. It was found that stalks inoculated with bacteria-free juice exhibited vascular discoloration at the lower parts of the nodes. Stalks out of the same setts that were not inoculated did not develop symptoms. Stalks of the non-inoculated setts were free of any vascular discoloration in the nodes.

Histological studies

Diseased and healthy mature stalks of sugar cane were studied histologically to determine the pathological effects induced by the ratoon-stunting virus. Sections through the nodal region of diseased stalks revealed the presence of unidentified material plugging the vessels (Figure 1 B). It also revealed the presence of necrotic cells in the phloem (Figure 1 C). Such pathological changes are confined to the net of the vascular bundles present at the lower part of the node. Healthy stalks do not, of course, exhibit such disorders (Figure 1A).

Hot-water treatment

In an attempt to control ratoon-stunting disease in field plantings of sugar cane, hot-water treatment developed by Mungomery [5] was adopted. An electric hot-water bath, thermostatically controlled to hold 120 lb of sugar-cane setts was used for treating the affected setts. A circulating water pump was attached to the bath to maintain the desired temperature throughout the water bath.

In order to establish a disease-free sugar-cane planting, setts of the N. Co. 310 and Co. 413 varieties which are grown commercially in the Kom Ombo area were selected for treatment. Recently cut mature stalks of each of these varieties were examined for the presence of internal symptoms of the disease. Only infected stalks were used for the hot-water treatment. To avoid any danger of injuring the buds by treatment, only the lower mature half of the stalks was used. The upper halves were reserved for planting as untreated controls. The lower halves were sectioned into cuttings, each of which contained three buds.

[5] Mungomery, R.W. 1953. Obstacles to be avoided in hot-water-treating cane setts against ratoon-stunting disease. *Cane Growers' Quart. Bull., Queensland.* 17:54-56.

Treatment began in March 1958; setts were immersed for 1½ hours at 52° ± 0.5°C. Each run contained 135 setts, totalling 405 buds. Temperatures usually dropped when setts were first immersed; it took about 15 minutes for the water to reach 52°C again. Setts, therefore, were always left in the hot-water bath for 1¾ hours. They were then removed and immersed for 30 minutes in a fungicidal bath containing ½ lb of 6 percent clerit in 20 gallons of water. The treated setts were then placed in jute bags and kept damp until planted either the same or the following day. Setts of each run were planted in nine rows within a plot measuring 7 × 7.5 meters. Setts taken from the nontreated upper half of the stalks were planted in a similar plot adjacent to the one with treated setts. This arrangement was repeated until 1 acre was planted, half of it with treated setts and the other half with nontreated setts. Half of the planting consisted of variety N. Co. 310, the other half of the variety Co. 413.

Results

Percentages of germination of the treated and nontreated setts in both N. Co. 310 and Co. 413 varieties were determined in May 1958, approximately two months after the heat treatment. It was found that 30.4 percent of the buds of the treated setts of N. Co. 310 germinated, whereas 41.8 percent in the nontreated setts germinated. In the variety Co. 413, about 27.9 percent germinated in the treated, and about 54.6 percent in the nontreated.

Examination of the mature cane took place in February 1959. About 12,000 stalks of the treated crop of Co. 413 were examined. The lower two nodes were examined for vascular discoloration. All stalks were found to be free from discoloration, except 13 that exhibited a vascular discoloration not typical of the ratoon-stunting disease. About 500 stalks of the treated N. Co. 310 were examined and all were found clean. Also, about 500 stalks of the nontreated plots of both varieties were examined and it was found that about 80 percent exhibited the typical vascular discoloration of ratoon stunting.

After examination, all the treated canes were planted in an area of about 6 acres.

In September 1959, the first ratoon of the treated N. Co. 310 and Co. 413 varieties was cut and the stalks examined. About 10,000 stalks of the treated N. Co. 310 variety were inspected and all were found free from the disease, except 13 which exhibited symptoms resembling the vascular discoloration caused by ratoon stunting. About 12,000 stalks of the treated Co. 413 variety were examined; all were found clean except for four suspicious plants. All the examined stalks were planted in an area of about 3 acres.

In February 1960, about 5,000 stalks of the crop of N. Co. 310 and Co. 413, planted in February 1959, were examined; none were found to exhibit the internal symptoms of the disease. The entire crop was distributed and planted in three localities in the Kom Ombo area as foundation plantings for further propagation.

Discussion

The occurrence and identification of ratoon-stunting virus disease in Egypt were confirmed by the success in transmitting and reproducing vascular discoloration in sugar-cane plants through the use of bacteria-free juice extracted from infected stalks and also by the success in controlling the disease by heat treatment.

Sugar-cane varieties grown commercially in Egypt do not last for many years. A named variety may be grown for several years and then abandoned and replaced by a new variety, due mainly to the deterioration of yield. In an area like Kom Ombo, four varieties have been cultivated successively during the last 20 years. These were P.O.J. 1030, Co. 281, Co. 2878, and Co. 413. The last-named is now being replaced by N. Co. 310. This deterioration in the productivity of established varieties and the need for frequent replacement take place in all sugar-cane-growing areas in the country.

The survey in the Kom Ombo area revealed that ratoon stunting is present in all the fields examined. Its prevalence suggests that this disease is probably one of the chief causes of yield deterioration of sugar-cane varieties, and

the attempts at establishing sugar-cane plantations free from ratoon stunting may soon elucidate its role in the deterioration of varieties N. Co. 310 and Co. 413.

As the result of the hot-water treatment, there are now in existence in the Kom Ombo region plantings of sugar cane free from ratoon stunting. These were set out as foundation plantings in five different localities in the Kom Ombo region to serve as a source of virus-free seed cuttings. At present, these foundation plantings consist of some 15 acres and will be under constant inspection to determine whether there is any natural spread of the disease in the field.

On the basis of the examination of about 40,000 stalks of treated cane, in the course of which only 30 suspiciously discolored stalks were found, the writers are inclined to think that vectors of the ratoon-stunting virus are not yet present in Egypt.

A program to establish ratoon-stunting-free sugar-cane plantings in all commercial areas is under way, in which large-scale heat treatment of setts will take place every year. Also, quarantine regulations have been formulated to subject all importations of sugar cane to a post-entry inspection period of not less than one year, during which time setts will be grown in quarantine greenhouses under insect-free conditions. The resultant crop will receive the hot-water treatment for 1½ hours at 52°C. Treated setts are to be planted outdoors for another year, after which the crop will be examined for presence of the disease. The healthy setts will then be released for planting in varietal trials. This procedure will allow the testing of newly imported varieties under virus-free conditions, provided of course that precautions will be taken to avoid transmission of the disease through cutting tools.

In the course of quarantine work on foreign varieties, it was noted that importations from Coimbatore, India, under numbers M. 165/38, H.M. 661, C.P. 29/320, C.P. 44/101, Co. K. 30, Co. 1193, Co. 1155, Co. 1141, Co. 1131, Co. 1129, Co. 1127, Co. 1095, Co. 1090, Co. 975, Co. 793, Co. 475, Co. 515, Co. 617, Co. 775, Co. 785, Co. 798, Co. 957, Co. 997, Co. 395, Co. 688, Co. 744, and Co. 782, were affected by vascular discoloration resembling that caused by the ratoon-stunting virus. The imported setts were planted under greenhouse conditions for one year. When canes from these setts were harvested, all varieties showed the characteristic discoloration of the disease. On the other hand, vascular discoloration was not noticed in canes of seven varieties which originated from setts treated by hot water before planting. However, canes of the first ratoon of these seven varieties did show symptoms suggestive of ratoon stunting.

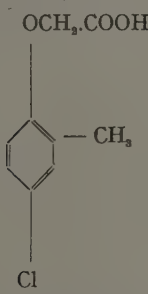
Recommended Analytical Methods for Pesticides

8. DETERMINATION OF MCPA BY THE USE OF LIQUID/LIQUID CHROMATOGRAPHY [1]

Collaborative Pesticides Analytical Committee,
c/o Plant Pathology Laboratory, Harpenden, Herts, England

This paper describes a method for determination of MCPA in the technical material, based on liquid/liquid chromatography. The method is suitable for MCPA and its formulations, but experience of this type of chromatography is essential before good results can be expected. Another method using infrared determinations will be published later.

MCPA is defined here as the recommended common name for 4-chloro-2-methyl-phenoxyacetic acid. Pure MCPA is a white crystaline solid.



Empirical formula $C_9H_9ClO_3$
Molecular weight 200.5

I. Technical MCPA

For analytical work a representative sample should be taken and should be not less than 100 gm.

[1] This method was accepted in November 1960 as a CPAC Method by the Collaborative Pesticides Analytical Committee (CPAC), which consists of scientists from ten European countries having a substantial chemical industry. The original method is in English. The early work of the MCPA Committee is described in Gardner, K. The Analysis of Dinoseb and MCPA Weedkillers. *Jour. Sci. Fd. Agr.* 7:8. 1956.

Determination of the MCPA content [2]

Principle

The four acids, 4,6-dichloro-2-methyl-phenoxyacetic, 4-chloro-2-methyl-phenoxyacetic, 6-chloro-2-methyl-phenoxyacetic and 2-methyl-phenoxyacetic acids are separated by partition chromatography, using kieselguhr and phosphate buffer as liquid static phase and a diethyl ether-chloroform mixture as the moving phase. Determinations of 4-chloro-2-methyl phenoxyacetic acid are carried out by titrating a predetermined fraction of the eluate with methanolic barium hydroxide.

Reagents

All reagents used shall conform to recognized analytical reagent quality, with the exception that British Pharmacopoeia specifications of solvent diethyl ether and chloroform are satisfactory.

Solution A (Note 1). Mix 112 ml. of 0.25M disodium hydrogen orthophosphate and 88 ml of 0.25M sodium dihydrogen orthophosphate.

Solution B. Mix equal volumes of diethyl ether and chloroform, cool to room temperature and keep protected from strong light.

Solution C (Note 2). Equilibrate solutions A and B at room temperature by shaking 1 liter of B with 50 ml of A in a separating funnel. Cool to room temperature. Filter the lower layer through cotton wool

[2] This method is based on that of Freeman, F. and K. Gardner. 1953. Determination of chloromethylphenoxyacetic acid in MCPA formulations. *Analyst* 78:205.

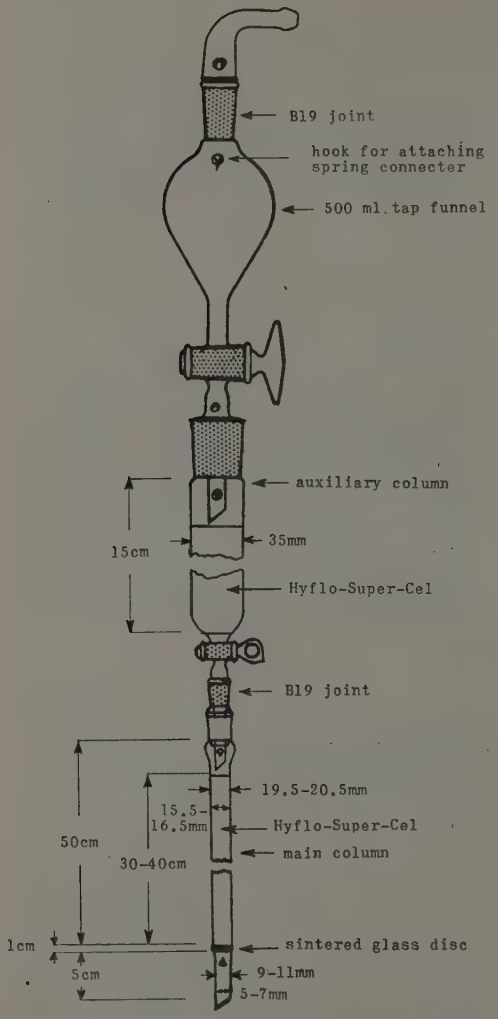


Figure 1. Chromatographic assembly for determination of MCPA. The various parts should be fitted with hooks for spring connectors.

to remove traces of suspended aqueous phase and retain as "moving phase."

Bromothymol blue, 0.04 percent in methanol

Barium hydroxide, 0.005N methanolic solution (Note 3)

Hyflo-super-cel (Note 4)

Cotton wool, extracted with diethyl ether

Diethyl ether

MCPA, pure

Filter paper, Whatman No. 1

Apparatus (Note 5)

Chromatography tube. This consists of a 1.55 to 1.65-cm inner diameter glass tube about 50 cm long, constricted at the lower end and with a B19 socket at the upper end. It should have a square-cut, not oblique end at the base (see Figure 1).

500-ml tap funnel, B19 cone and socket

Sinter glass disc, porosity No. 2

Auxiliary column about 15 cm long, 3.5-cm diameter, fitted with a B19 cone at the lower end and a B19 socket at the upper end (see Figure 1).

Packer stainless steel disc 1 mm less in diameter than the column inner diameter, 4 mm thick, provided with six 1.5-mm holes and a centrally located rod 3-mm diameter and about 60 mm long

Microburette (5 or 10 ml) calibrated in 0.01 or 0.02-ml divisions (Note 6)

1-ml pipette, to deliver between two graduations

15-ml graduated pipette

100-ml volumetric flask

Cylinder of nitrogen or carbon dioxide-free air

600-ml beaker

25-ml measuring cylinder

250-ml measuring cylinder

Glass-stirring rod, ½-in diameter

Forty 6-in × $^{8}/_{8}$-in test tubes

Twelve 100-ml conical flasks

Method

(*a*) Preparation of the column (Note 7)

Place 25 gm of Hyflo-super-cel in a 600-ml beaker and add dropwise with careful mixing 12.5 ml of the buffer solution A. A ½-in glass rod serves as a convenient stirring device and reduces the tendency to grind the Hyflo-super-cel. Triturate for ten minutes. Add 200 ml of the equilibrated solvent mixture C and again triturate gently for four minutes (Note 8).

A sintered glass disc is placed in the constricted part of the tube and two circles of filter pa-

per placed on top of the disc. The tube is loosely clamped in the vertical position. It is convenient to place one clamp below the constriction and another near the top of the column. The tube can now be rotated easily but will stand firm under vertical pressure. Pour the slurried Hyflo-super-cel into the tube, and pack the first 0.5 cm of the column relatively firmly to produce a sound base for the column.

From a position 3 to 4 cm above the packed surface, gently lower the packer disc so as to compress 1 to 2 mm of the material. Rotate the tube, and with short strokes, using minimum pressure, consolidate the edge of the column. Repeat this process until the whole of the Hyflo-super-cel has been packed in 1 to 2-mm sections.

The consolidation of the edges of the column after each section is packed is most important. Large amounts of thick slurry must not be pushed down and impacted quickly, as this leads to air entrainment in the column. The column should be slowly and uniformly built up, each addition to slurry with subsequent packing resulting in a uniformly packed column, the whole operation taking at least one hour. The length of the packed column should be 30 to 40 cm. Should the length of the packing not fall in this range, then either the trituration or the packing has not been done correctly. Longer and more vigorous trituration and/or harder packing will produce a shorter column (Note 9).

Throughout the packing operation a head of solvent should be maintained above the packed solid. The solvent running from the lower end of the column should be collected in a beaker and returned to the slurry as and when required.

To prolong the life of the column, fit an additional small auxiliary column above the normal column (see Figure 1). The lower end of the auxiliary column is packed with ether-extracted cotton wool and the body of the column is almost filled with the slurried Hyflo-super-cel prepared as described above. The Hyflo-super-cel is packed into a relatively firm column, using the main column stainless steel packer. The packing is, of course, not critical (Note 10). Using this auxiliary column, over 40 analyses have been carried out on one-column packing.

(*b*) Standardization of the column (Note 11)

The column need not normally be standardized against pure phenoxyacetic acids, but an analysis should be carried out on a technical sample. If the base line is not reached between each component, the column is rejected and repacked with fresh material. The method of standardization is described under *c* (*ii*).

(*c*) Determination of MCPA in the sample

(*i*) *Preparation of sample.* Weigh out sufficient of the sample (*w* gm) to contain 500 to 800 mg of 4-chloro-2-methyl phenoxyacetic acid into a 100-ml volumetric flask dissolved in diethyl ether; make up to the mark with diethyl ether and mix well.

(*ii*) *Chromatographic separation and titration of 4-chloro-2-methyl phenoxyacetic acid.* Assemble the apparatus as shown in the diagram, fitting the auxiliary column and the tap funnel, both of which should be filled with solution C. Apply nitrogen pressure and pass some 200 ml of solution C through the column. The pressure required to give the recommended flow rate of 2 to 2.5 ml per minute should not be more than 5 pounds per square inch gauge. Should the pressure required be very much greater than this, the column has probably been packed too tightly (Note 9). Remove the auxiliary column and separating funnel (Note 10). Allow the level of the liquid in the main column to fall until the layer of liquid above the Hyflo-super-cel is very thin.

Carefully pipette 1 ml from the volumetric flask onto the column. The pipette delivering between two graduations should be used for this purpose. Force this liquid onto the column, using a pressure of nitrogen until the 1 ml is just absorbed. Follow the same procedure with two successive 1-ml portions of solution C.

Fill the column with solution C and replace the auxiliary column and separating funnel. Always take care not to trap air bubbles at

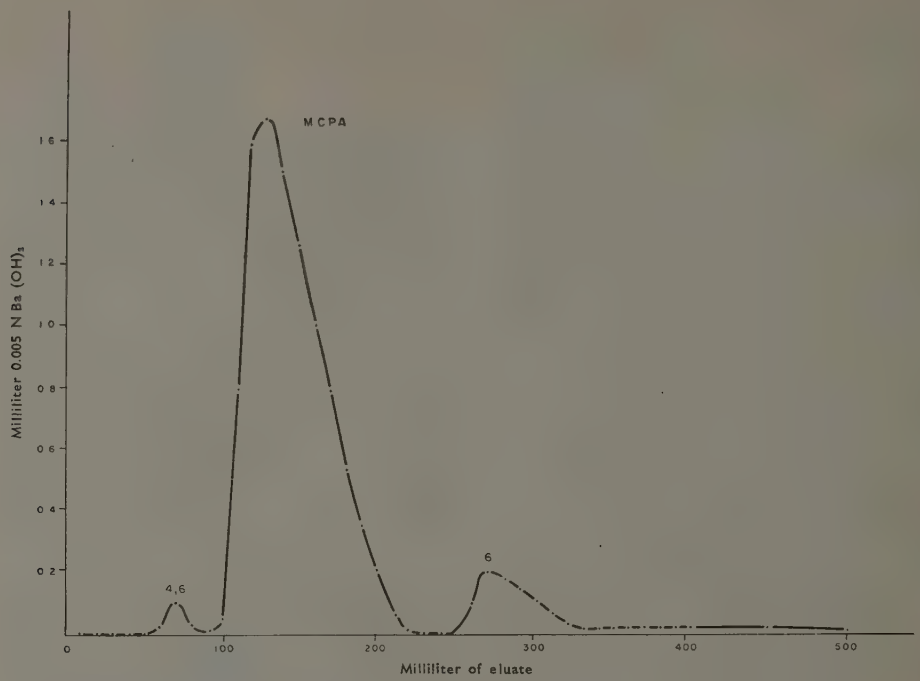


Figure 2. Chromatogram from a column which has been used 40 times (4.6 = 4.6-dichloro-2-methyl phenoxyacetic acid, MCPA = 4-chloro-2-methyl phenoxyacetic acid, 6 = 6-chloro-2-methyl phenoxyacetic acid).

the joints when replacing the auxiliary column and funnel.

Elute the column with 1 liter of solution C (Note 12).

In the case of the first run, collect 10-ml fractions over the first 400 ml, and 50-ml fractions thereafter. Pass a stream of nitrogen through each fraction for three to four minutes and titrate with the barium hydroxide solution to the first end point of the bromothymol blue indicator. Ensure that the same volume of indicator per volume of solution (0.1 ml per 100 ml of solution) is used throughout. Determine the solvent blank and correct the individual titers accordingly (Note 13).

From the titers obtained, plot a graph of volume of eluate against titrations. An example of a typical curve is given in Figure 2. If the troughs between the peaks are not well defined (Note 14), the column is unsuitable for use.

Provided a satisfactory curve is obtained (Note 15), the column may be run " blind " at the same flow rate, the first analysis on the column indicating where each component appears. Future fraction sizes can then be modified accordingly and the appropriate MCPA fraction collected over the predetermined eluate volume (Note 16). It is advisable to check the column after four extractions and after storage (Notes 10 and 17).

If the total net titer of the 4-chloro-2-methyl phenoxyacetic acid fractions is t ml of 0.005N alkali, then :

$$\text{Percentage w/v of 4-chloro-2-methyl phenoxyacetic acid} = \frac{20.06\, t}{w}$$

Note 1. Use recrystallized sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) and *di*-sodium hydrogen orthophosphate ($Na_2HPO_4.12H_2O$). As the percentage of water of crystallization in these salts varies, approximately

0.25M solutions are made up and standardized.

Weigh 23.0 gm of *di*sodium hydrogen orthophosphate ($Na_2HPO_4.12H_2O$) and 10.0 gm of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$). Dissolve each salt in distilled water (recently boiled out and cooled) and make up each of the two separate solutions to 250 ml in volumetric flasks.

Titrate 25 ml of the *di*sodium hydrogen orthophosphate solution with 0.25N hydrochloric acid, using three drops of 0.4 percent bromocresol green solution as indicator. Match the color at the end point to that of 25 ml of the sodium dihydrogen orthophosphate solution containing three drops of the bromocresol green solution. Hence calculate the exact amount of *di*sodium hydrogen orthophosphate needed to give a 0.25M solution in 250 ml of water. Make up the solution and finally check the strength by titrating as before with 0.25N hydrochloric acid.

Similarly, titrate 25 ml sodium dihydrogen orthophosphate solution with 0.25N sodium hydroxide solution, using three drops of 0.04 percent thymol blue solution as indicator. Match the color at the end point to that of 25 ml of the *di*sodium hydrogen orthophosphate solution containing three drops of thymol blue solution. Hence calculate the exact volume of water to be added to give a 0.25M solution and dilute accordingly. Make up the solution and finally check the strength by titrating as before with 0.25N sodium hydroxide solution.

Prepare the buffer solution by mixing 112 ml of exactly 0.25M *di*sodium hydrogen orthophosphate and 88 ml of exactly 0.25M sodium dihydrogen orthophosphate solutions, using a Class A burette to measure the volumes, and mix well. All phosphate solutions should be stored in polythene bottles at 0°C to prevent chemical contamination and mold growth. Always shake the bottle before use.

An alternative procedure is to dissolve 3.975 gm anhydrous *di*sodium hydrogen orthophosphate (Na_2HPO) and 3.433 gm hydrated sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) in distilled water (recently boiled out and cooled) and dilute to 200 ml. The purity of the phosphates should be determined as described above.

NOTE 2. A suitable volume of diethyl ether and of chloroform should be brought into equilibrium with buffer and blank titers determined before the mixed solvent is made up for chromatography, so that any imperfection in either solvent can be rectified before large quantities of mixed solvents are prepared.

It is preferable to use freshly prepared solution B, as preferential evaporation of the ether may take place on storage.

NOTE 3. 0.005N methanolic barium hydroxide solution is prepared and standardized as follows:

Dissolve barium hydroxide (0.8 gm $Ba(OH)_2.8H_2O$) in methanol and dilute to 1 liter.

Dissolve about 0.2 gm, accurately weighed, of pure MCPA in chloroform and dilute to exactly 100 ml with chloroform. Take a 5-ml aliquot either (preferably) by weight *or* by measurement from a microburette, taking care to avoid evaporation losses during transference of this solution into a dry 100-ml flask, add equilibrated solution C (20 ml), pass a stream of carbon dioxide-free nitrogen through the solution for three to four minutes, and add 10 drops (0.2 ml) of the bromothymol blue solution. Titrate directly with the barium hydroxide solution to the first end point of bromothymol blue. Carry out a blank using the solvents only, and subtract the titer obtained from the initial titer.

Barium hydroxide solution should be stored in a bottle protected by a soda asbestos guard tube. This will reduce the rate of deterioration and frequency of standardization.

NOTE 4. "Hyflo-super-cel" is obtainable from Johns-Manville Ltd., Artillery Row, London, S.W. 1, and should be dried at 105°C four hours before use.

NOTE 5. All volumetric glassware must be of Class A standard.

NOTE 6. The burette should be protected with a soda asbestos guard tube.

NOTE 7. The personal factor plays an important role in the successful packing of a column. An analyst must acquire a technique for packing columns, and this may even necessitate minor modification of the procedure.

NOTE 8. The mixed solvent should be brought into equilibrium with the buffer immediately before use.

NOTE 9. It is preferable to have a column length near 40 cm, but columns down to 25 cm have given satisfactory results. With the shorter columns, higher pressures of nitrogen will be needed to maintain a flow rate of 2 to 2.5 ml. Higher gas pressures may result in loss of efficiency of the column, due to excessive degassing.

NOTE 10. The auxiliary column must always be kept full of solution C and precautions must be taken to seal the lower end when the column is detached from the assembly. This column serves to ensure that the solution C is equilibrated with buffer before passing through the main column. The main column should never be allowed to dry out.

NOTE 11. The column can be standardized, if required, by using the following solution D instead of the commercial sample.

Solution D

Dissolve

4.6-dichloro-2-methyl phenoxyacetic acid, melting point[3] 188°C (0.1 gm)

[3] Sjoberg, B. 1950. Determination of chloro-2-methyl phenoxyacetic acid by infrared spectrophotometry. *Acta. Chem. Scand.* 4:798-805.

4-chloro-2-methyl phenoxyacetic acid, melting point 120°C (0.5 gm)
6-chloro-2-methyl phenoxyacetic acid, melting point 109°C (0.2 gm)
2-methyl phenoxyacetic acid, melting point 155°C (0.2 gm)
in solution B 100 ml

NOTE 12. The use of about 1 liter of eluant will ensure that the 6-chloro-2-methyl phenoxyacetic acid and unchlorinated acids are removed from the column, which is then ready for re-use.

NOTE 13. A blank of 50 ml of solvent passed through the column should not normally exceed 0.04 ml of 0.005N barium hydroxide.

NOTE 14. The 10-ml intermediate fractions should have titers of less than 0.03 ml.

NOTE 15. Nonuniform packing of the Hyflo-super-cel will produce a poor separation of the acids, and variation of the buffer salt concentrations from those required will affect the resolution and position of the peaks.

NOTE 16. The two fractions immediately before and after this volume of eluate should be collected and the four 10-ml fractions titrated to check that a satisfactory separation has been achieved.

NOTE 17. Columns which have been standing for more than two days should be flushed out with about 50 ml of freshly equilibrated solvent before use.

DETERMINATION OF THE FREE PHENOLS CONTENT AS 4-CHLORO-2-METHYL PHENOL (Note 1)

Principle

The sample is dissolved in alcohol; ammonia solution, 4-aminophenazone, and potassium ferricyanide solutions added and the optical density determined with a photoelectric absorptiometer, or spectrophotometer.

Reagents

4-chloro-2-methyl phenol, 0.01 percent w/v solution; dissolve 100 mg of the phenol in 10 ml acetone and dilute to 1 liter with distilled water.
MCPA, 0.1 percent w/v solution of the phenol-free acid (Note 2) as its ammonium salt; dissolve 1.0 mg of the acid in 50 ml ethanol, add 90 ml 0.05N ammonia solution, and dilute to 1 liter with distilled water.
Ammonia solution, 0.05N.
4-aminophenazone hydrochloride, 0.2 percent w/v aqueous solution prepared as required from a 2 percent stock solution. The stock solution can be stored in the dark for periods of up to three months.
Potassium ferricyanide, 0.4 percent aqueous solution, freshly prepared.

Apparatus

Photoelectric absorptiometer or spectrophotometer (Note 3)
2-ml microburette
Three 5-ml pipettes
10-ml pipette
Eight 25-ml stoppered measuring cylinders
100-ml measuring cylinder
1,000-ml measuring cylinder

Method

(*a*) Preparation of the calibration samples

Transfer, using the microburette 0.4, 0.8, 1.0, 1.2, 1.6, 2.0, 2.5 and 3.0-ml portions of the phenol solution into separate 25-ml stoppered measuring cylinders, and make up the volume in each to 10 ml with MCPA solution. Pipette ammonia solution (5 ml) into each cylinder, mix the contents, add 4-aminophenazone solution (5 ml) and mix again. Finally, add potassium ferricyanide solution (5 ml) to each cylinder, shake vigorously, and after five to ten minutes measure the optical density of the solutions in a 1-cm cell, using distilled water in the reference cell (Note 4).

Determine a blank on the reagents by taking 10 ml of the MCPA solution and treating with ammonia solution, 4-aminophenazone, and potassium ferricyanide solutions as above. Subtract the blank reading from the readings obtained on the phenol solutions. Prepare a calibration curve for the instrument, plotting optical density against ml of the phenol solution.

(*b*) Determination of free phenols in the sample

Weigh out 1.0 gm of the sample, dissolve it in ethanol (50 ml), and add ammonia solution (90 ml). Make up the volume to 1 liter with distilled water. Pipette 10 ml of this solution

into a 25-ml stoppered measuring cylinder and add, in turn, ammonia solution (5 ml), 4-aminophenazone solution (5 ml), hydrochloride solution (5 ml), and potassium ferricyanide solution (5 ml), shaking after each addition (Note 4).

Continue shaking for about one minute after the addition of the ferricyanide solution, and measure the optical density of the solution after five to ten minutes. Prepare a "blank" as described under "Preparation of calibration sample," and deduct the blank from the value obtained with the sample. Hence determine, from the calibration curve, the percentage of 4-chloro-2-methyl phenol present.

1 ml solution A = 1.0 percent 4-chloro-2-methyl phenol in the original sample.

NOTE 1. The method is suitable for the determination of 0.1-3.0 percent 4-chloro-2-methyl phenol in MCPA samples.

NOTE 2. For the preparation of pure MCPA, see Sjoberg, *loc. cit.*

NOTE 3. Hilger "Spekker" absorptiometer with an Ilford 604 filter.

NOTE 4. It is important that the reagents be added in the order stated.

SOLUBILITY OF SALTS IN WATER

Reagent

Sodium hydroxide, 0.5N solution

Apparatus

30-ml stoppered test tube
10-ml measuring cylinder

Method

Weigh out 1 gm of the sample, transfer to the test tube, and dissolve the sample in the sodium hydroxide solution (10 ml) by shaking. The material should yield a solution free from appreciable sediment.

DETERMINATION OF SULPHATED ASH

Determine the sulphated ash on 5 gm of the sample, as follows:

Reagents

Ethanol, 95 percent, or industrial methylated spirit
Sulphuric acid

Apparatus

Tared silica crucible, 51 mm × 51 mm or larger

Method

Place the specified amount of the sample in the tared crucible (x gm) and reweigh (y gm). Moisten well with ethanol, and add concentrated sulphuric acid (5 drops). Heat gently on a gauze or asbestos board until evaporated to dryness. This preliminary evaporating should take about one to two hours, to avoid sputtering. Then ignite it until most of the carbon is burned off; allow to cool, add a few drops of concentrated sulphuric acid, and re-ignite to constant weight (z gm).

Percentage of sulphated ash (w/w)

$$= \frac{100 \times (z-x)}{y-x}$$

II. Salts of MCPA and formulations

containing metallic or amine salts of MCPA

MCPA CONTENT

Reagents

Hydrochloric acid, 1 + 1
Diethyl ether

Apparatus

Two 250-ml separating funnels
50-ml measuring cylinder
100-ml volumetric flask

Method

Weigh sufficient sample (w gm) to contain about 500 to 800 mg of 4-chloro-2-methyl phenoxyacetic acid. Dilute with water (to about 50 ml) and acidify with hydrochloric acid (1 + 1). Extract with two portions of diethyl ether (2 × 30 ml). Wash the combined ether layers with three successive por-

tions of water (3 × 10 ml). Wash the combined water washings with diethyl ether (30 ml) and add to the main ether solution. Transfer the combined ether layers to 100-ml volumetric flask, make up to the mark with diethyl ether and mix well. Continue by the method described under "Chromatographic separation and titration of 4-chloro-2-methyl phenoxyacetic acid" for technical MCPA.

Percentage 4-chloro-2-methyl phenoxyacetic acid (w/w) $= \frac{20.05\ t}{w}$

$$(w/v) = \frac{20.05\ t.s}{w}$$

where t = total titer of 4-chloro-2-methyl phenoxyacetic fractions of ml of 0.005N barium hydroxide

s = specific gravity at 60°/60° F

DETERMINATION OF PHENOLS CONTENT AS 4-CHLORO-2-METHYL PHENOL

Determine the phenols content by the method described under "Determination of the free phenols content as 4-chloro-2-methyl phenol" for technical MCPA

III. MCPA esters (technical)

MCPA CONTENT

Reagents

Ethanol, industrial methylated spirit
Hydrochloric acid, 1 + 1
Diethyl ether
Sodium hydroxide, 5N approximately

Apparatus

100-ml conical flask fitted with ground glass joint
Reflux condenser to fit the flask
10-ml measuring cylinder
100-ml measuring cylinder
Three 250-ml separating funnels
100-ml volumetric flask

Method

Weigh sufficient MCPA ester sample to contain 500 to 800 mg of 4-chloro-2-methyl phenoxyacetic acid (w gm) into the conical flask. Dissolve in industrial methylated spirit (10 ml) and add sodium hydroxide solution (3 ml). Reflux for 30 minutes. Cool, and dilute to about 60 ml with distilled water. Acidify with hydrochloric acid and extract with diethyl ether three times (1 × 30 ml, 2 × 20 ml). Wash the combined ether layers with three successive portions of water (3 × 10 ml). Wash the combined water washings with diethyl ether (30 ml) and add to the main ether solution. Transfer the combined ether layers to the volumetric flask, make up to the mark with diethyl ether and mix well. Continue by the method described under "Chromatographic separation and titration of 4-chloro-2-methyl phenoxyacetic acid" for technical MCPA.

Percentage 4-chloro-2-methyl phenoxyacetic acid (w/w) $= \frac{20.05\ t}{w}$

$$(w/v) = \frac{20.05\ t.s}{w}$$

where t = total titer of 4-chloro-2-methyl phenoxyacetic fractions in ml of 0.005N barium hydroxide

s = specific gravity at 60°/60° F

NOTE 1. With some preparations difficulties may be encountered as a result of emulsion formation during extraction. This difficulty can often be overcome by adding an inorganic salt to the emulsion.

OUTBREAKS AND NEW RECORDS

CANADA

Wolf G. Ziller, Forest Entomology and Pathology Laboratory, Victoria, B.C.

Monterey and bishop pine susceptible to sweetfern blister rust

During the past decade, approximately 200 experimental plantations of exotic forest trees have been established in the Province of British Columbia. In the course of an examination of these plantations in the spring of 1961, severe outbreaks of the sweetfern blister rust disease, caused by *Cronartium comptoniae* Arth., were noted among five-to-eight-year-old pine seedlings. Approximately 40 percent of the 9,000 Monterey pine (*Pinus radiata*) and 20 percent of the 3,000 bishop pine (*P. muricata*) examined were found infected with the rust. In one plantation the infection reached 95 percent. Other species of pine growing in the same plantations, notably Austrian pine (*P. nigra*), cluster pine (*P. pinaster*), and the hybrids *P. echinata* X *taeda*, *P. banksiana* X *contorta* and *P. rigida* X *taeda* were completely free from sweetfern blister rust, although *P. banksiana*, *P. contorta*, *P. echinata*, *P. pinaster*, *P. rigida*, *P. taeda*, as well as at least nine other species of two- and three-needle pines are known to be susceptible. It is to be noted that many of these susceptible but healthy pine seedlings grow near or directly alongside the heavily rusted Monterey and bishop pines. These observations, recently reported by Molnar,[1] constitute first records of *P. radiata* and *P. muricata* as hosts of *Cronartium comptoniae*.

Apparently the pines did not become infected in the nursery but at the plantations. Large areas covered with sweet gale (*Myrica gale*), a telial host of *Cronartium comptoniae*, were discovered within one-half mile of most of the severely rusted pines, whereas no telial hosts were present in the vicinity of the nursery where the pines were raised.

Figure 1. Stem of six-year-old *Pinus radiata* almost girdled by *Cronartium comptoniae*. The peridia have ruptured and most of the aeciospores have shed.

Cronartium comptoniae (Figure 1) is difficult to distinguish from *Peridermium stalactiforme* Arth. & Kern, another blister rust of two- and three-needle pines restricted to North America. To ascertain the identity of the parasite as it occurs on pine, its telial state was obtained by controlled inoculations. Aeciospores of the rust were used to inoculate In-

[1] Molnar, A.C. 1961. An outbreak of *Cronartium comptoniae* on Monterey and bishop pines on Vancouver Island, British Columbia. *Plant Disease Reptr.* 45:854-855.

dian paint brush (*Castilleja* sp.) as well as sweet gale, the former a common telial host of *P. stalactiforme*. The results, total lack of infection of Indian paint brush and heavy infection of sweet gale, confirmed the rust on Monterey and bishop pine as being *Cronartium comptoniae*.

Cronartium comptoniae, like *C. ribicola* J.C. Fischer, is a heterocecious rust parasite unable to survive without telial hosts (Myricaceae) in the vicinity of susceptible pines. Hansbrough[2] described the disease caused by sweetfern blister rust as follows: "The disease damages by its girdling action and the smaller the tree the more likely that complete girdling will occur and that death will ensue... The spread of this disease to and from the pines and the alternate hosts is by windborne spores... Spread of the disease into disease-free regions is largely through shipment of infected pine nursery stock."

Cronartium comptoniae is to be considered a potentially dangerous parasite, particularly if it should become established in parts of the world like Australia and New Zealand, where Monterey pine comprises an important part of the forest economy.

[2] Hansbrough, J.R. 1940. The sweetfern blister rust of pitch pines. *U.S. Dept. Agr. Tree Pest Leaflet* 45.

KENYA

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Armyworm outbreak in 1961

The armyworm, *Spodoptera* (= *Laphygma*) *exempta*, occurs in Kenya in outbreak proportions at approximately two-year intervals. The caterpillars may be present in the active phase between November and June but never in the period from July to October.

Under normal conditions, the outbreak moves north from Tanganyika, arriving in southern Kenya in January and moving north into the cereal-growing areas in late March, April and May. Damage is confined to grasses and cereals, although cutworm-type damage is sometimes reported in crops of beans, lucerne and cotton. Vast grazing areas are affected, often at a time when the new flush of grass would be of value to domestic livestock. Broadleaved weeds and sedges are left undamaged, leading to a marked deterioration in the value of pasture. Selective feeding may result in some grasses being severely damaged, and their recovery is slow. In 1961 this slow recovery was intensified by drought conditions. In cultivated areas, maize, sorghum and millet are affected. These may be eaten down to below ground level and, while recovery sometimes occurs, successive waves of caterpillars often prevent it.

The year 1960 was a year of low rainfall for much of Kenya, though there was enough fodder to maintain cattle throughout the dry season from December to April. In 1961, the armyworm outbreak, expected in January, did not occur until the end of March, when conditions were dry and hot. The period of high temperatures extended throughout the rainy season, which again had rains of below average in April and May. Livestock farmers were then faced with a shortage of grazing just when fodder stocks were exhausted. This resulted in heavy losses in cattle, particularly in the Masai country, while milk and meat production was severely curtailed.

Armyworm moths, normally limited by temperature to a maximum altitude of 6,500 to 7,000 feet, were able to go higher during these hotter conditions, and caterpillars occurred up to 8,300 feet. The area of high potential agricultural districts invaded by armyworm was thus greater than usual and, because of drought conditions, the damage resulting was abnormally severe.

A broadcast warning of the invasion led to immediate action in the European farming areas and many infestations of small caterpillars were detected before they could cause much damage. Boom spraying from tractors was widely used, generally with good results. It soon became apparent, however, that supplies of insecticide were inadequate and that the best materials for armyworm control were not always available. Insecticides of low persistency gave good kills initially but failed to give crop protection for more than a few days; spraying had therefore to be repeated and some fields were sprayed three times. Some farmers, unable to obtain insecticides in time, had to resign themselves to replanting. This happened in many African districts where neither insecticides nor spraying machines were obtainable. Aircraft were used whenever feasible, and these were so effective that a saving of insecticide was possible by reducing rates of application. Thus the standard recommendation of 3 pints of 25 percent DDT per acre or of 1½ pints of dieldrin emulsifiable concentrate, was reduced to 2 pints and 1 pint respectively.

Shortage of these materials led to the use of endrin, aldrin, gamma BHC, malathion, diazinon and toxaphene. Dipterex gave an excellent kill, as did pyrethrum formulations, but had not sufficient persistence to prevent reinfestation. Further supplies of DDT were brought in by air. Parathion was not used because of its high mammalian toxicity, but its use was contemplated had supplies of other insecticides run out. Cattle dip formulations were extensively used, but low-volume application was discouraged in case scorch damage should occur.

Where invasion of maize had taken place from surrounding grassland, the spraying of a zone around the edge of the field was often adequate, but where grass contours are employed, the infestations usually arose within the field and the central area had to be sprayed.

Early in the outbreak, death of the armyworm was reported due to a nuclear polyhedrosis, and a supply of the virus from such dead caterpillars was collected. A suspension of the polyhedral crystals was made simply by adding the decomposing bodies to water. A rough basis of ten caterpillars to a gallon of water was used, and this foul-smelling liquid was sprayed from aircraft, boom sprayers and motorized knapsacks. The latter were particularly suitable, as low-volume rates of about 1 gallon of spray per acre could be employed. As latent virus may have been present throughout the infested areas, there was no proof that the spraying of virus produced all the results achieved. In all areas, however, where young caterpillars were sprayed with virus, dead bodies filled with virus could be found seven to ten days later. Where large caterpillars were sprayed, pupation took place before symptoms were produced. In one area, death from a bacterial disease occurred within two days of spraying virus. Once the virus was well established in the field, it spread through infested grassland, where little or no chemical control had been practiced. This suggests that an additional build-up had taken place from a latent supply of virus in the soil. No such build-up occurred in crops which had been sprayed with insecticides. The outbreak thus ended in sprayed crops, for there was no reinfestation.

As the virus was used by many farmers, its success was universally acclaimed. Considerable biological control was, however, achieved by various Tachnids, and the Eulophid, *Euplectrus laphygmae*, was recorded in Kenya for the first time. The outbreak ended, as did that of 1959, with enormous numbers of moths which did not breed. The reason for this was not known, though it was popularly believed that they had been sterilized by the virus.

The possibility of reducing the severity of future outbreaks by the use of virus deserves consideration. Virus undoubtedly occurs in soil or in the bodies of related caterpillars, slowly builds up during the outbreak, and is responsible, in part, for its cessation. Areas probably occur where there is no virus, and there a reduced armyworm population survives and reverts to the passive phase. If virus could be stored from one outbreak to the next and sprayed on the first available concentra-

tions of armyworm, then the natural build-up of this disease would be accelerated. By inter-territorial action, this might prevent outbreaks originating, say, in Tanganyika, from spreading northward into Kenya and Uganda. It is, however, scarcely possible for the territorial entomologists, to whom armyworm is only one of the many pests to be controlled, to achieve much in this direction.

The successes gained by the international control of locusts suggest that armyworm, a pest from South Africa to Ethiopia, might similarly be controlled by interterritorial efforts with the aid of special staff.

PLANT QUARANTINE ANNOUNCEMENTS

FRANCE

A Ministerial Order of 29 September 1961, published in the *Journal officiel*, Vol. 93, No. 238, on 10 October 1961, authorizes the importation into the metropolitan customs area of France (including Corsica) of any living plant, grafting material or unrooted cuttings of the Rosaceae family, if the consignments satisfy the following conditions :

(*a*) Plants or parts thereof must have been officially inspected in the field during the growing period and found to be free from fire blight (*Erwinia amylovora*).

(*b*) Consignments must be accompanied by a certificate in the form of that annexed to the International Plant Protection Convention of 1951, with the additional declaration, " La réglementation phytosanitaire française est respectée. "

GILBERT and ELLICE ISLANDS

The Prohibition (Importation of Plants) Order, 1960, published on 16 June 1960, concerns importation of plants from certain Pacific Islands. The Order repeals all prohibitive and restrictive measures imposed under provisions of the Plants Ordinance.

1. Importation of all plants from the following places is absolutely prohibited : American Samoa and the Trust Territory of Western Samoa ; Wallis Islands and adjacent small islands ; Horne Islands (Fortuna and Alofa) ; The Kingdom of Tonga and the Island of Niue.

2. Importation of all plants from Fiji is prohibited, unless (*a*) they are accompanied by a fumigation certificate issued by the Department of Agriculture in Fiji ; (*b*) they have only been in transit through Fiji and stored in a refrigerated condition ; (*c*) if an authorized officer is satisfied that the plants are apparently free from pests or diseases.

IRAQ

Law No. 27 for Importation of Plants of 27 March 1961, published in *The Weekly Gazette of the Republic of Iraq* No. 33 on 16 August 1961, replaces the Importation of Plants Law No. 31 of 1938.

General requirements

1. All plants and parts of plants, whether living or desiccated, including roots, bulbs, tubers, stems, leaves, flowers, fruits, seeds and lints, arriving in the country by any means of transportation will be examined by plant quarantine officers. All imported plants, even if found free from pests or diseases, will be fumigated, washed or dressed before release.

2. The plant quarantine officers may order the consignments destroyed if they were found infested to a degree incurable by disinfestation or otherwise fail to meet the required sanitary conditions, unless they are re-exported within seven days.

3. These restrictions do not apply to fruit juices and preserved, dried, pickled or crystallized fruits.

Imports prohibited or restricted

1. Importation and transit of the following is prohibited, except those imported by the agricultural departments for trial purposes :

(*a*) Citrus fruits of all varieties.

(*b*) Vine plants, parts thereof, and fruits.

(*c*) Seed cotton.

2. Cotton seed and its waste require approval by the High Committee of Provisions.

3. Living or dead insects of any species require an import license and prior approval by the Ministry of Agriculture.

LUXEMBOURG

1. Ministerial Rule of 13 October 1961, published in the *Memorial* A No. 45 on 31 October 1961, concerns importation of seed potatoes for the 1962 growing season. Merchants are authorized to import seed potatoes only if they have previously purchased locally produced seed potatoes of approved standards, at the proportion of 1.5 local potatoes for each potato to be imported.

Seed potatoes of the following grades are admitted for importation: Class E, A and B of the varieties Bintje and Eersteling, and Class E, A and "Hochzucht" of all other officially approved varieties. Limited quantities of other varieties may be imported for experimental purposes.

2. Ministerial Rule of 21 October 1961, published on the same date, supersedes Decree of 18 February 1898 concerning importation of living plants and fresh fruit from America and the Ministerial Decrees of 24 September and 20 October 1947 concerning measures to prevent introduction and spread of San Jose scale.

Importation of living San Jose scale is prohibited, except under a special authorization issued by the Ministry of Agriculture for scientific purposes only.

Importation of living woody plants and parts thereof, including fruits, is permitted only if the consignments are accompanied by a phytosanitary certificate issued by the plant protection service of the country of origin, attesting that the San Jose scale does not occur in the country or that the shipment has been examined and found free from San Jose scale. Seeds, cut flowers and underground parts are exempted from this restriction.

Consignments of living woody plants not accompanied by the prescribed certificate will be placed under the control of the Plant Protection Service, and if found infested by San Jose scale, they will be sent back or destroyed.

Living woody plants or parts thereof, except scions of the following genera, may be imported only from 10 October to 15 April: *Acer, Cotoneaster, Crataegus, Cydonia, Euonymus, Fagus, Juglans, Ligustrum, Malus, Pyrus, Populus, Prunus, Ribes, Rosa, Salix, Sorbus, Syringa, Tilia, Ulmus.*

Plants belonging to the genera enumerated above, and parts thereof (except cut flowers not intended for multiplication), will be fumigated upon arrival with hydrocyanic acid or another approved fumigant. Evergreen plants of these genera may be exempted from fumigation if they were found free from San Jose scale. Shipments subject to fumigation should be addressed to the fumigation station at Luxembourg Ville.

Transit shipments of living woody plants, parts thereof and fruits are not subject to phytosanitary measures, if their packing conditions will prevent the escape of pests. Otherwise they will be inspected by the Plant Protection Service.

UNITED KINGDOM (England and Wales, Scotland)

The Importation of Potatoes (Amendment) Order, 1961, which came into operation on 10 July 1961, amends the Importation of Potato Order 1959 (see *FAO Plant Prot. Bull.* 7 : 81-82, 1959) by adding the Delta Area of the United Arab Republic (Egypt) between latitude 30° 20 N. and 31° 30 N. to the list of countries from which main crop potatoes may be imported into England and Wales.

The Importation of Plants (Scotland) Amendment Order 1961 amends the Importation of Plants (Scotland) Order 1955 (see *FAO Plant Prot. Bull.* 3 : 143, 1955 ; 8 : 51, 1960) and contains the same provisions as in the Order for England and Wales.

Stabilimento tipografico FAUSTO FAILLI - Via Tuscolana, 128, Roma

In preparation

MANUAL OF FUMIGATION FOR INSECT CONTROL

FAO Agricultural Studies No. 56

Stressing the simpler, more readily adaptable techniques used in fumigation for control of insects above the ground, this 290-page manual is written essentially for the practical fumigator and for officials who supervise or conduct quarantine treatments. Every effort has been made to present the principles underlying the use of fumigants in a clear, concise manner, so that the manual serves as a ready guide for operators.

In addition to principles, the volume deals with precautions and protective devices, modern fumigants, space and vacuum fumigation, fumigation of grain, standing trees and crops, greenhouses, plants, vegetables, fruit, flowers, mills, empty structures, etc. Plant quarantine treatments are discussed, as are fumigation measures for the control of rodents, snakes, ants, wasps and termites.

The manual, which is amply illustrated, will be a useful reference for senior planners and consultants in crop protection who require information on the scope and limitations of fumigation as an instrument for insect control. Also of interest is a section dealing with general first aid for accidents with fumigants.

On publication (scheduled for September-October 1962), this study will be available in English, French and Spanish editions through local FAO sales agents or from the Distribution and Sales Section of FAO in Rome, Italy.

$3.00 or 15s.

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